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## **AMENDMENTS TO THE CLAIMS**

Please amend the specification pursuant to 37 C.F.R. 1.121 as follows:

- (Original) A method for estimating a melting temperature (T<sub>m</sub>) for a polynucleotide at a desired ion concentration [X<sup>+</sup>], said polynucleotide having a known G-C content value, f(G-C), comprising:
  - (a) obtaining a reference melting temperature  $(T_m^{\ 0})$  for the polynucleotide, said reference melting temperature being a melting temperature obtained or provided for the polynucleotide at a reference ion concentration  $[X^+]_0$ ; and
- (b) modifying the reference melting temperature by a logarithm of the ratio of said

  desired ion concentration to said reference ion concentration, said logarithm being

  multiplied by a coefficient which is a function of the G-C content value,

  wherein the estimated melting temperature is calculated using the reference melting

  temperature.
- (Original) A method for estimating a melting temperature (T<sub>m</sub>) for a polynucleotide at a desired ion concentration [X<sup>+</sup>], said polynucleotide having a known G-C content value, f(G-C), comprising:
  - (a) obtaining a reference melting temperature  $(T_m^{\ 0})$  for the polynucleotide, said reference melting temperature being a melting temperature obtained or provided for the polynucleotide at a reference ion concentration  $[X^+]_0$ ; and
  - (b) modifying the reference melting temperature by an amount,

$$k(f(G-C)) \times \ln \frac{[X^+]}{[X^+]_0}$$

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in which the coefficient k(f(G-C)) is a function of the G-C content value f(G-C), wherein the estimated melting temperature is obtained by using the reference melting temperature.

3. (Original) The method of claim 2, wherein the coefficient k has a value determined by the relation

$$k(f(G-C)) = m \cdot f(G-C) + k_0$$
; and

wherein a first coefficient, m and a second coefficient,  $k_0$ , are optimized for predicting polynucleotide melting temperatures  $T_m^0$ .

4. (Original) The method of claim 2, wherein the reference melting temperature  $T_m^{\ 0}$  is used to calculate  $T_m$  according to the formula:

$$T_m = T_m^0 + k \times \ln \frac{[X^+]}{[X^+]_0}$$

5. (Original) The method of claim 4, wherein the coefficient k

$$k(f(G-C)) = m \cdot f(G-C) + k_0$$
;

and wherein a first coefficient, m and a second coefficient,  $k_0$ , are optimized for predicting polynucleotide melting temperatures  $T_m^0$ .

6. (Currently amended) The method of claim 2, wherein the reference melting temperature  $T_m^0$  is used to calculate  $T_m$  according to the formula:

$$T_m = T_m^0 + k (f(G - C)) \times \ln \frac{[X^+]}{[X^+]_0} + b \times (\ln^2 [X^+] - \ln^2 [X^+]_0)$$

wherein a coefficient b is optimized for predicting polynucleotide melting temperatures.

- 7. (Original) The method of claim 6, wherein k is  $m \cdot f(G-C) + k_0$ ; and wherein a first coefficient, m, a second coefficient,  $k_0$  and a third coefficient b are optimized for predicting polynucleotide melting temperatures  $T_m^0$ .
- 8. (Original) The method according to claim 5, wherein m is -3.22,  $k_0$  is 6.39.
- 9. (Original) The method according to claim 7, wherein m is -4.62,  $k_0$  is 4.52 and b = -0.985.
- 10. (Original) The method of claim 2, wherein the reference melting temperature  $T_m^0$  is used to calculate  $T_m$  according to the formula:

$$\frac{1}{T_m} = \frac{1}{T_m^0} + k(f(G - C)) \times \ln \frac{[X^+]}{[X^+]_0}.$$

- 11. (Original) The method of claim 10, wherein the coefficient k has a determined value by the relation  $kf(G-C) = m \cdot f(G-C) + k_0$ ; and wherein a first coefficient, m and a second coefficient,  $k_0$  are optimized for predicting polynucleotide melting temperatures.
- 12. (Currently amended) The method of claim 2, wherein the melting temperature is obtained from the reference  $T_m^0$  by utilizing the formula:

$$\frac{1}{T_m} = \frac{1}{T_m^0} + k (f(G - C)) \times \ln \frac{[X^+]}{[X^+]_0} + b \times (\ln^2 [X^+] - \ln^2 [X^+]_0)$$

wherein a coefficient b is optimized for predicting polynucleotide melting temperatures.

- 13. (Original) The method of claim 10, wherein k is  $m \cdot f(G-C) + k_0$ ; and wherein a first coefficient, m and a second coefficient,  $k_0$ , and a third coefficient b are optimized for predicting polynucleotide melting temperature.
- 14. (Original) The method of claim 11, wherein  $k_0$  is  $-6.18 \times 10^{-5}$ ; m is  $3.85 \times 10^{-5}$ .
- 15. (Original) The method of claim 13, wherein  $k_0$  is  $-3.95 \times 10^{-5}$ ; m is  $4.29 \times 10^{-5}$ ; and b is  $9.40 \times 10^{-6}$ .
- 16. (Original) The method of claim 2, wherein the G-C content value is the fraction of the polynucleotide's nucleotide bases that are either guanine or cytosine.
- 17. (Original) The method of claim 1, wherein the polynucleotide is DNA.
- 18. (Original) The method of claim 1, wherein the polynucleotide ranges in length from about 2 to about 500 basepairs.
- 19. (Original) The method of claim 1, wherein the polynucleotide ranges in length from about 5 to about 200 base pairs.
- 20. (Original) The method of claim 1, wherein the polynucleotide ranges from about 10 to about 30 basepairs in length.
- 21. (Original) The method of claim 1, wherein the reference melting temperature is experimentally determined.

22. (Original) The method of claim 1, wherein the reference melting temperature is calculated from a theoretical model.

- 23. (Original) The method of claim 1, wherein the reference melting temperature is obtained by utilizing a nearest neighbor model.
- 24. (Original) The method of claim 1, wherein the reference ion concentration is 1 M.
- 25. (Original) The method of claim 1, wherein the ion is a monovalent ion.
- 26. (Original) The method of claim 1, wherein the ion is selected from the group consisting of the cations of sodium, lithium, potassium, rubidium, cesium and francium.
- 27. (Original) The method of claim 1, wherein the desired ion concentration ranges between about 1mM and about 5M.
- 28. (Original) The method of claim 1, wherein the desired ion concentration ranges between about 10 mM and about 2M.
- 29. (Original) The method of claim 1, wherein the desired ion concentration ranges between about 70 mM and about 1021mM.
- 30. (Original) A computer system for predicting a melting temperature, which computer system comprises:
  - (a) a memory; and

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(b) a processor interconnected with the memory and having one or more software components loaded therein,

wherein the one or more software components cause the processor to execute steps of a method according to claim 1.

31. (Original) A computer program product comprising a computer readable medium having one or more software components encoded thereon in computer readable form, wherein the one or more software components may be loaded into a memory of a computer system and cause a processor interconnected with said memory to execute steps of a method according to claim 1.